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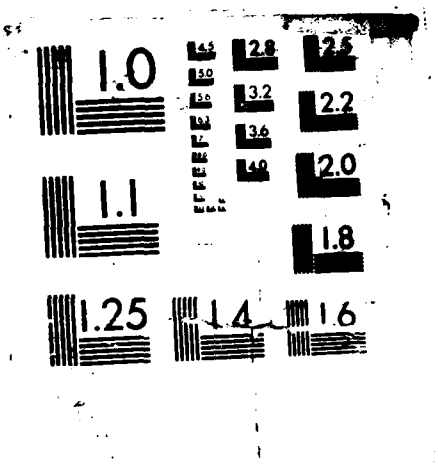

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Kraeger, Sping J. Gennevois, Danial J.P., Cox, R. A., and Karu, Alexander E..

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This note summarizes recent observations on the antigenic specificity and suitability for diagnostic use of seven IgM monoclonal antibodies (MAbs) prepared in 1984 with c. immitis Silveira spherules and endospore/ shperule culture filtrate (ECSF) as immunogens.

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RECOGNITION OF *COCCIDIOIDES IMMITIS* ANTIGENS WITH MONOCLONAL ANTIBODIES

S.J. Kraeger[1], D.J.P. Gennevois[2], R.A. Cox[3], and A.E. Karu[2]

[1]University of California, School of Public Health, Berkeley, CA; [2]Naval Biosciences Laboratory, Oakland, CA; [3]San Antonio Chest Hospital, San Antonio, TX

This note summarizes recent observations on the antigenic specificity and suitability for diagnostic use of seven IgM monoclonal antibodies (MAbs) prepared in 1984 with *C.immitis* Silveira spherules and endospore/spherule culture filtrate (ECSF) as immunogens.

Nature of the Antigens. All seven MAbs reacted with a heat-stable methanol-precipitable extract of *C.immitis* endospores, suggesting that the antigenic determinants include carbohydrate. Denaturing electrophoresis and immunoblotting of ESCF resolved protein-containing bands with apparent molecular weights of 66, 65, 56, 45, and 32 kDa that were recognized by MAbs E35 and S82, suggesting shared epitopes. Coccidioidomycosis patient sera reacted with these same bands. The same bands bound fluorescein-labeled concanavalin A (conA), but no other lectins. Competition between conA and E35 suggested that glucose or mannose is close to, or part of the epitope of E35. Non-denaturing gel filtration resolved the antigens in ESCF into a broad distribution of sizes which showed three separate patterns of reactivity with the different MAbs (Figure 1). Deproteinized extracts of the largest carbohydrate-containing antigens (fractions 21-38 and 42-43 in Figure 1) were too complex for magnetic resonance analysis, but fractions 55 and 56, which bound only MAbs E35 and S82, contained a single oligosaccharide of B-D-glucose.

Specificity of the Antibodies. Only two MAbs showed more than 20% cross-reactivity with the other major systemic fungi in radio- and enzyme immunoassays (RIA,EIA). All of the MAbs reacted with spherules of six different *C.immitis* strains and ESCF from the Silveira strain, but four MAbs did not react with the ESCF from strain 46, which is of low virulence. Fluorescence microscopy showed specific differential staining of spherules, hyphae, and arthroconidia by the MAbs. For example, Figure 2 shows staining of the ends of arthroconidia by MAb E36. The MAbs also bound differently to these morphologic forms in RIAs.

Diagnostic Applications. All seven MAbs reacted with the *C.immitis* skin-testing antigens and the mycelial lysates that are currently used to detect precipitin-forming and complement-fixing antibodies. Experiments are under way to determine whether any of the antigens are diagnostic for coccidioidomycosis. We have developed a latex agglutination (LA) test to detect *C. immitis* antigens; to date the detection limit is 1 ng of *C.immitis* ESCF at 20 ng/ml in buffer. Detection thresholds for other pathogenic fungi are

summarized in Table 1. We are attempting to optimize sensitivity and specificity when sera are tested by this method.

In summary, the determinants recognized by all seven MAbs appear to be primarily carbohydrates, found on several biochemically separable species. Each MAb reacted differently with various antigens or fungal particles in EIA, RIA, immunofluorescence, and LA tests. MAbs in this panel detect taxonomic and developmental differences in isolates of *C. immitis* and other pathogenic fungi, and some can be used to localize antigens on the surface of fungal cells. Some of these MAbs are uniquely specific for *C. immitis* antigens, and their potential usefulness in diagnostic assays for coccidioidomycosis deserves further study.

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Fig. 1. Sephacryl S-400 gel filtration profile of *C. immitis* ESCF. The top panel shows the protein and carbohydrate content of each fraction. The succeeding panels show the reactivities of three MAbs with equivalent amounts of carbohydrate from each column fraction in an EIA. The carbohydrate profile is reproduced in each panel for reference. V_o is the void volume.

Fig. 2. Fluorescence micrograph of *C. immitis* arthroconidia reacted with MAb E36 and fluorescein-labeled goat anti-mouse globulin, showing differential staining of the arthroconidia. Magnification X 594.

Table 1. Minimum detectable dilutions of culture filtrates (ng of carbohydrate) in a microplate LA test. Polystyrene beads (0.3 um) coated with the indicated MAbs were mixed with culture filtrates diluted in 0.05ml of 0.85% NaCl-0.05 M glycine-NaOH (pH 8.2) with 0.1% bovine serum albumin, in microtiter "U"-shaped wells. Agglutination was scored after incubating the plates overnight at 22 C.

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